Marine biology

Development in juvenile Weddell seals: Diving, physiology, nutritional status, and survivorship

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This project examined the physiological development of Weddell seal pups relative to changes in diving ability, nutritional status, and survivorship. In the austral summer of 1994, the study consisted of three main parts:

- an intensive study of the regulation of blood metabolites and heart rate during diving, sleep apnea, and eupnea in pre- and postweaned pups;
- further monitoring of morphometrics and blood chemistry profiles related to health and nutritional status in a subsection of the pup population; and
- a continuation of the population parameter studies of the Weddell seals in Erebus Bay.

Diving physiology

It has been shown previously that Weddell seal pups begin to dive within 2 weeks of birth, are accomplished divers by 6 weeks of age, and continue to develop their diving abilities over several years (Burns and Testa in press). Little is known, however, about their physiological development during the same period. To improve understanding of the limitations faced by Weddell seal pups during the critical period around weaning, an in-depth study was conducted using the isolated hole dive protocol first developed by Kooyman (1968, pp. 227–261). Two main hypotheses were tested:

- that the ability of pups to regulate their blood chemistry and heart rate during apnea (breath holding), eupnea (breathing), and diving would develop with age and
- that the development of the physiological control necessary for diving would occur in concert with their behavioral development.

To test these hypotheses, four pups (37–50 days old) were taken from the colony at Big Razorback Island in McMurdo Sound, Antarctica (166.48°E 77.684°S) to a remote site where they were catheterized and equipped with heart rate monitoring leads. The experimental protocol allowed the pattern of change in hematocrit, plasma lactate, glucose, heart rate, and vasoactive hormones to be studied during diving and recovery, sleep apnea, and eupnea. In each pup, the apneic heart rate was lower than the eupneic rate, and postdive levels of hematocrit, glucose, and lactate were correlated with the length of the dive; however, although the preweaned pup was unable to regulate its apneic heart rate consistently and showed little pattern in postdive hematocrit or lactate levels, the weaned pup regulated both heart rate and blood chemistry in a fashion similar to that of adults. This change in control ability was also reflected by a dramatic increase in the pups' aerobic dive limit during the period around weaning (Castellini 1995; Burns and Castellini in press).

In the young Weddell seal pups, as in other mammals, respiration was associated with higher heart rate, angiotensin II and arginine vasopressin levels, and lower atrial natriuretic peptide levels. Changes in the circulating concentrations of these vasoactive hormones were clearly linked to heart rate variability during bouts of eupnea and apnea. These results suggest that in Weddell seal pups, the ability to regulate the cardiovascular system develops early and that the vasoactive hormones maintain cardiovascular integrity differently during apnea and eupnea (Zenteno-Savin and Castellini in preparation). Overall, these findings support the hypotheses that Weddell seal diving physiology and behavior develops rapidly and that by the time the pups need to forage on their own, they have both the behavioral and physiological maturity necessary to survive.

Health and juvenile survivorship

As in the previous season, a large subset (32 percent) of the pup population was captured once near weaning so that pup health (blood metabolites) and body condition (mass, standard length, and axillary girth) could be assessed. The long-term goal of this study is to correlate information on the health and condition of pups at weaning with later survivorship. Understanding this relationship, however, will require another 5 to 6 years of census data before survivorship rates can be accurately estimated.

Although juvenile survivorship has not yet been linked with condition at weaning, the effects of birth year, age, and sex on survivorship have been tested for using the mark-recapture data from 1973 to 1994. Juvenile survivorship estimates average 45 percent from birth to age 1, 65 percent from age 1 to 2, and 80 percent from age 2 to 6. In most seasons, pup sex, weaning weight, and survivorship were influenced by maternal characteristics. In years of poor adult female reproductive success, the birth and survival rate to age 1 was lower

for male, but not for female, pups. In addition, pups born to younger and/or smaller mothers had lower weaning weights and lower pre- and postweaning survivorship (Hastings 1996).

Population dynamics study

To monitor the Weddell seal population in Erebus Bay, six weekly censuses were conducted during the summer. Mark-recapture estimates indicated a total (nonpup) population size of 213±16 (standard error) adult males and 564±13 adult females. Adult tag proportions were maintained at 50 percent for males and 58 percent for females. All pups (377) were tagged near birth. The total number of pups born in 1994 was similar to the number of births the previous two seasons (349 in 1992 and 417 in 1993), and there was no indication of a significant change in total population size.

Three trips were made to White Island to tag and take blood samples from all newborn pups and untagged adults in the small Weddell seal population there. Mark-recapture analysis methods estimate the White Island population to be fewer than 25 adult animals, but genetic analyses have not revealed significant inbreeding depression (Fleischer, Perry, and Testa 1995). Paternity analysis is ongoing.

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Antarctic seabird ecology and demography in Admiralty Bay, King George Island, Antarctica

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Our field team arrived at the Copacabana Field Station in Admiralty Bay, King George Island, on 6 October 1995, to continue the long-term ecological and demographic studies of the region's penguin and flying bird populations. Brash ice lined the beach and fast ice filled the inlets, while the center of the bay was ice free. Polish scientists at nearby Arctowski Station reported that pack ice had covered the bay all winter and had moved out only a few days before our arrival (members of XX Polish Expedition personal communication). The snow had melted from the penguin breeding areas and both Adélie and gentoo penguins were attending pebble nests.

Our research had four main objectives:

- to monitor the reproductive success and demography of Adélie (*Pygoscelis adeliae*), gentoo (*P. papua*), and chinstrap (*P. antarctica*) penguins; south polar (*Catharacta maccormicki*) and brown skuas (*C. lonnbergi*); southern giant petrels (*Macronectes giganteus*); kelp gulls (*Larus dominicanus*); and American sheathbills (*Chionis alba*);

- to sample diets of the three *Pygocselis* penguin species during the chick-rearing phase;
- to monitor weight fluctuations in Adélie penguins throughout the breeding season; and
- to monitor leopard seal presence and behavior at the Copacabana colony.

We monitored penguin reproductive success and demography by following all known-aged birds plus a random sample of 150 nests each of Adélie and gentoo penguins throughout the breeding season. For the reproductive study, 100 penguins of each study species were banded, and the remaining 50 pairs served as a study control. Daily observations indicated that both the Adélie and gentoo penguins began egg laying in the third week of October. Egg-laying synchroneity was high for both species and peak egg laying occurred between 27 and 31 October. In comparison to 1994, these peak dates were a week earlier for Adélie penguins and two weeks earlier for gentoo penguins. The advanced, highly synchronous egg-

laying dates were most likely prompted by the early availability of snow-free nesting ground. Adélie penguin first incubation shifts averaged 11 days for males and 8 days for females, similar to previous years' data. Breeding success was high, with both species fledging more than one chick per pair. Population censuses indicated that the number of Adélie penguins breeding at the Copacabana colony increased slightly, whereas gentoo and chinstrap penguin numbers declined slightly from 1994. The large number of banded, known-age Adélie penguins sighted throughout the season indicated that recruitment of young Adélie penguins was high.

Chinstrap penguins arrived late in October, and egg laying occurred in the third week of November, consistent with previous years. Chinstrap penguins also fledged approximately one chick per pair, indicating that the 1995–1996 season was a productive breeding year for all three *Pygoscelis* species.

Brown skuas arrived and began setting up territories in early November, followed by south polar skuas approximately 2 weeks later. Reproductive success was slightly higher for brown skuas than for south polar skuas, though they both averaged one fledged chick per pair. South polar skua diets were sampled through guano collection at their nesting territories. Analysis of fish otoliths in the guano during courtship, egg laying, and chick rearing stages indicates that *Electrona antarctica* and *Pleurogramma antarcticum* were the dominant fish prey. Krill (*Euphausia superba*) was the other dominant prey item. We also monitored the reproductive success and demography of southern giant petrels, kelp gulls, and American sheathbills. No American sheathbills bred successfully near the field site this season; however, kelp gull and southern giant petrel nest numbers remained consistent with 1994. Kelp gulls fledged less than two chicks per pair and giant petrel reproductive success remained high, averaging 0.75 chicks fledged per pair.

Our second study objective was to quantify the diets of the three *Pygoscelis* penguin species, during the chick-rearing stage, using the water off-loading technique (Wilson 1984). Independent samples of five penguins per species per week were lavaged over 6 weeks to determine the weights and contents of the stomachs. Only penguins with active nest sites containing chicks were sampled. *E. superba* was the dominant prey item found in all three of the penguin diets. A random sample of 50 *E. superba* from each stomach were measured and sexed. A length-frequency analysis of *E. superba* size depicted a binomial distribution of both smaller/younger (16–30-millimeter) and larger/older (46–65-millimeter) animals. These data indicate the abundance of both young and old cohorts in the penguin diets and support the krill super cohort hypothesis (Trivelpiece and Trivelpiece in press) predicted for 1995–1996.

Our third objective at Copacabana Field Station was to continue the Adélie penguin weight study by monitoring weight fluctuations in breeding Adélie penguins throughout the season. The penguins used were those that had been banded and followed for the previous year's reproduction study. Each penguin was monitored daily to record dates and weights for arrival; egg laying; incubation shifts; chick hatching; and first, second, and third week of chick rearing. This study provides data on arrival weights, weight gain/loss, and incubation shifts that can be used to index foraging success and food availability throughout the season and over the years.

Our final objective was to monitor leopard seal behavior and predation events at the Copacabana penguin colony. We wanted to identify both biotic and physical variables that might influence leopard seal presence, activity, and foraging success. Observations were made from a blind 15 meters above Admiralty Bay, 50 meters from the beach. Leopard seal and penguin activity was recorded during 3-hour observation periods that rotated sequentially from dawn till dusk throughout the austral summer. Atmospheric and oceanic conditions were measured and continuous searches were made for leopard seals and penguin predation events. The number of leopard seal sightings and predation events were low during the 1995–1996 summer. Preliminary analysis indicates that leopard seal activity was more strongly correlated to the presence of pack ice in Admiralty Bay than to penguin activity at the Copacabana colony.

Whale sightings collected opportunistically throughout the season indicated that the numbers of whales sighted in Admiralty Bay were below the 10-year mean for orca (*Orcinus orca*), humpback (*Balaenoptera novaeangliae*), and minke (*Balaenoptera acutorostrata*) whales. Weddell (*Leptonychotes weddellii*) and crabeater (*Lobodon carcinophagus*) seals were present throughout the season, though not abundant. Fur seals (*Arctocephalus gazella*), which typically arrive around mid-January in large numbers, were delayed until early February. A number of flying bird vagrants also occurred in Admiralty Bay this season including yellow-billed pintail (*Anas georgica*), white-rumped sandpiper (*Calidris fuscicollis*), pectoral sandpiper (*C. melanotos*), and black-necked swan (*Cignus melancoryphus*).

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Growth and development in antarctic fulmarine petrels

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As part of a 3-year comparative study of the foraging ecology and reproductive energetics of four species of antarctic fulmarine petrels, we have been investigating chick growth and development. The overall aim of the study is to develop a better understanding of the population-level energetics in Prydz Bay through an investigation of adult foraging strategies, parental reproductive effort, and nestling energy requirements and how environmental variation affects reproductive performance. This research is providing us with insights into predator-prey dynamics in the antarctic marine ecosystem and the role and impact of fulmarine petrels as top-level predators.

Nestling growth rate is thought to reflect variations in rate and quality of food delivered (Croxall 1984, pp. 533–616). This, in turn, is assumed to be correlated with food availability. Deviations in nestling growth rate from allometric predictions should correspond to interspecific differences in food availability, parental effort, and/or nestling energy demand.

The primary field site is Hop Island (68°53'S 77°50'E) in the Rauer Island group, approximately 40 kilometers south-southwest of Australia's Davis Station. We arrived on station on 22 October 1995 and began the field season on the island on 29 October. We departed the island on 31 March 1996. During the 1995–1996 season, we studied diet composition, chick energetics and growth, adult energetics, and breeding success in populations of snow petrel (*Pagodroma nivea*), cape petrel (*Daption capense*), antarctic petrel (*Thalassoica antarctica*), and antarctic fulmar (*Fulmarus glacialoides*). The extended nature of the field season allowed us to follow all four species through the entire breeding season, from pre-breeding attendance through to fledging.

Known-age chicks were measured every 3 days throughout the nestling period from the day of hatching. These measurements continued until the chicks fledged. Logistic growth curves were fitted to the growth data for each species. The four petrel species studied grew markedly more rapidly than was predicted for petrels and albatrosses by an allometric equation derived by Croxall and Gaston (1988) (figure 1). Growth rate, in grams per day, was 169 percent of predicted for cape petrels, 125 percent of predicted for snow petrels, 165 percent of predicted for antarctic petrels, and 180 percent of predicted for antarctic fulmars. Mean adult masses are 469 grams (g) for cape petrels, 258 g for snow petrels, 687 g for antarctic petrels, and 859 g for antarctic fulmars. The nestling periods for cape petrels, snow petrels, antarctic petrels, and antarctic fulmars were 51 percent, 58 percent, 48 percent, and 48 percent, respectively, of the predicted time to fledge based on the predictive equation of Warham (1990).

We used open-circuit respirometry to determine resting metabolic rates of adults and chicks of all four species and to assess chick thermoregulatory capacity. The oxygen consumption of chicks was determined at six ages (3, 8, 15, 28, 35, and 42 days). Figure 2 presents data for 3-day-old and 8-day-old antarctic petrel chicks and illustrates the typical pattern with resting metabolic rate (RMR) increasing as chicks grew and with the thermoneutral zone shifting to lower temperatures. The lower critical temperature (T_{lc}) of chicks of cape petrel, southern fulmar, and antarctic petrel was initially near 20°C but fell to near 0°C by 28 days age. Snow petrel chicks were less tolerant of low temperatures with T_{lc}s about 10°C or higher at all ages. Our data permit us to characterize fully the resting metabolic rates of fulmarine petrel chicks throughout the nestling phase.

Growth and respirometry research was augmented by doubly labeled water (DLW) measurements of field metabolic rate (FMR) on nestlings of all four species. We measured FMR of chicks at seven intervals throughout the nestling period. Adult FMR was determined on incubating snow and cape petrels and on snow, cape, and antarctic petrel adults during the chick-rearing period.

Diet samples were collected using the water off-loading technique (Wilson 1984). All four species were sampled at two intervals during the chick period. Preliminary analysis of the samples indicates that diet was similar to the preceding two seasons and was composed of fish (almost exclusively *Pleuragramma antarcticum*) and krill (*Euphausia superba* and *E. crystallorophias*). Snow petrels fed predominantly on fish (approximately 83 percent fish by mass), cape petrels fed
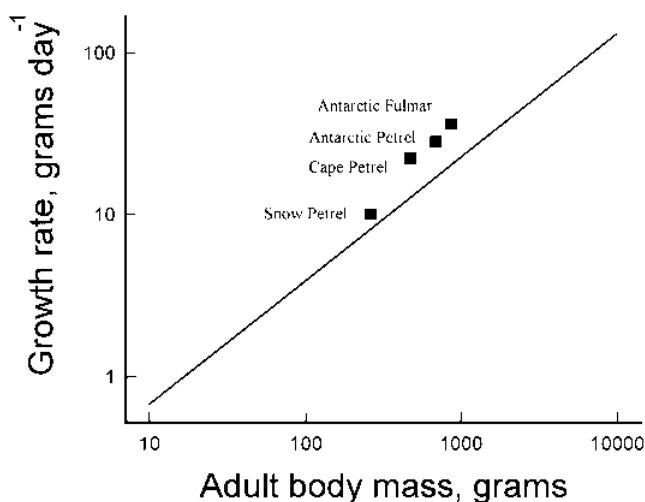

Figure 1. Relation of growth rate of four species of antarctic fulmarine petrels (boxes) to regression line derived from allometric equation in Croxall and Gaston (1988) as follows: $y=0.118x^{0.76}$ where x=adult body mass in grams and y=growth rate in grams per day.

primarily on krill (70 percent krill by mass), and antarctic fulmars and antarctic petrels were intermediate in their prey preference (63 percent krill by mass and 40 percent krill by mass, respectively).

Diet was also quantified using stable isotope analysis [delta carbon-13 (^{13}C) and delta nitrogen-15 (^{15}N)] of fecal samples, blood samples, eggshell fragments, and feathers. This technique relies on differential incorporation of isotopes in organisms foraging at different trophic levels. We are investigating the utility of the technique for long-term, minimally invasive dietary monitoring programs.

Different patterns of growth between fulmarine and nonfulmarine procellariiforms reflect different ecological constraints on breeding. Time available for breeding appears to be the primary constraint for antarctic breeding species whereas food is often cited as the primary constraint in more temperate regions. Nestling growth rates are relatively rapid for antarctic fulmarine petrels, suggesting abundant resources and possibly the constraint of a short season in which environmental conditions appropriate for breeding are limited.
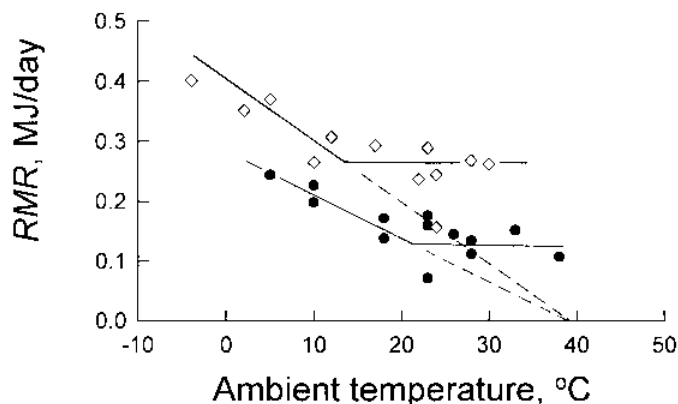
Figure 2. Relation of resting metabolic rate (RMR) of 3-day-old (dots) and 8-day-old (diamonds) antarctic petrel chicks to ambient temperature. (MJ denotes millijoule.)

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Phenotypic plasticity in the nototheniid fish *Trematomus newnesi* from McMurdo Sound, Antarctica

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The common nototheniid fish *Trematomus newnesi* has a circumantarctic distribution in cold, shallow shelf waters of the continent and adjacent islands (DeWitt, Heemstra, and Gon 1990, pp. 279–331). Although known from ice-covered McMurdo Sound since early in the century, *T. newnesi* has been infrequently collected from ice holes over deep water. During the 1991–1995 field seasons, however, fishing in water about 20 meters deep near McMurdo Station yielded a large sample of this species. Although conforming to the taxonomic description of *T. newnesi*, 19 of 67 specimens (28 percent) were separable as a distinct form: a large-mouth/broad-headed morph. This is the first report of phenotypic plasticity in any marine fish, and it provides insight into the nature of antarctic fish diversity at the intraspecific or population level.

The large-mouth morph was distinct and separable by eye over the full size range of the sample. [Large-mouth morphs were 116–239 millimeters (mm) standard length; 27–258 grams.] All specimens could be designated as either the large-mouth or typical morph by inspection of head shape and relative width and by size and position of the mouth (figures 1 and 2). The head was U-shaped, and the upper and lower jaws were larger and heavier in the large-mouth morph. In addition, the gape angle of the closed mouth was at a greater angle from the horizontal in the large-mouth morph than in the typical morph (figure 1). The large-mouth morph was significantly longer (total length) and heavier than the typical morph. Furthermore, nine of 14 morphometric measurements were significantly greater in the large-mouth morph. The large-mouth morph had a longer, deeper, and wider head; larger gape and upper jaw; longer pelvic fins; and a deeper caudal peduncle. These differences were also reflected in proportions relative to head and standard lengths, with significant differences in jaw and snout length and body depth and a nearly significant difference in caudal peduncle depth. Dissection of a number of other trophic, sensory, and osteological structures showed no differences between the
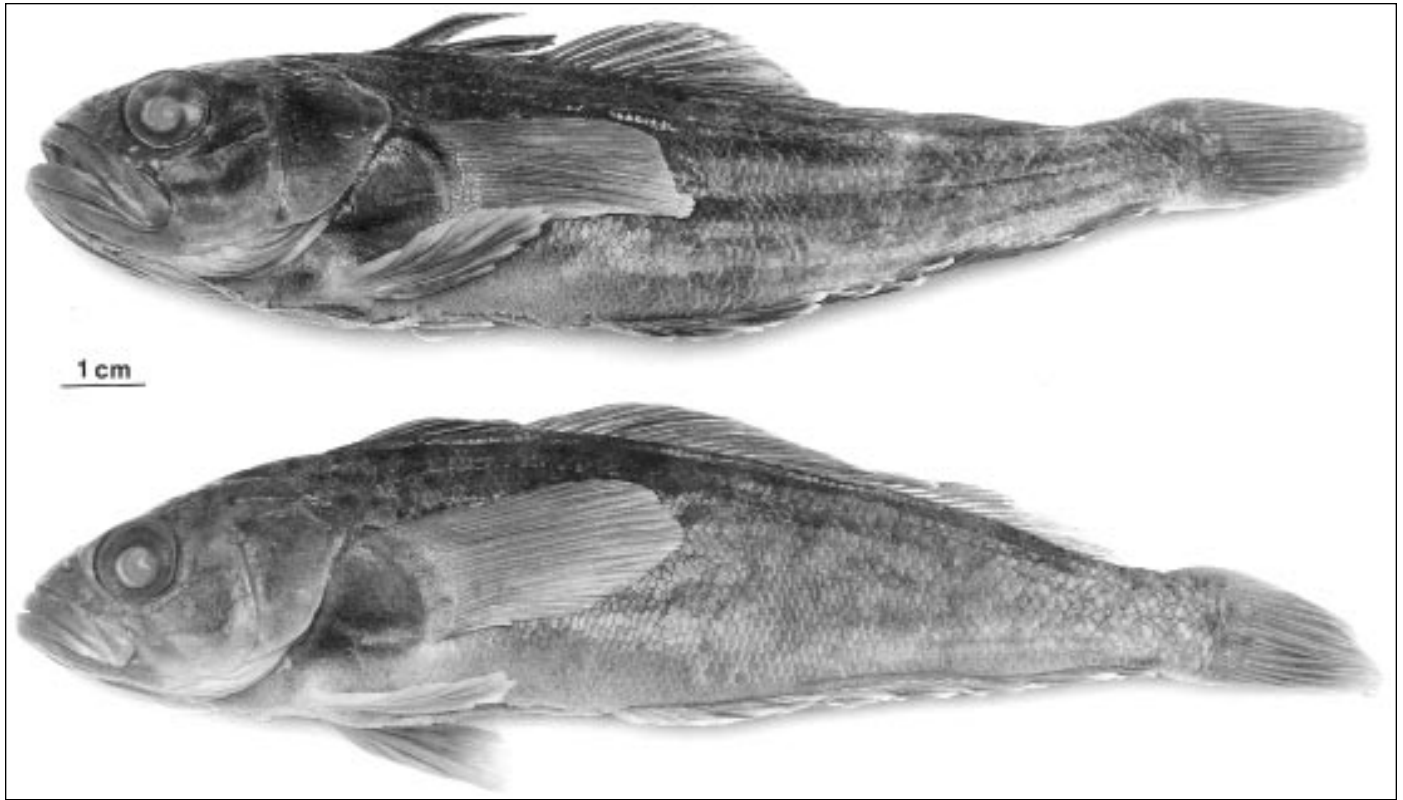
Figure 1. Morphological comparison of lateral aspect of similarly sized morphs of *Trematomus newnesi* from McMurdo Sound. Large-mouth/broad-headed morph on top and typical morph on bottom. Blunt snout, size and angle of the mouth, and position of upper jaw relative to eye are shown. The large-mouth morph is a male measuring 162 mm standard length and weighing 42.7 grams. Typical morph is a female measuring 164 mm standard length and weighing 47.5 grams. (× 1.4)

two morphs. The background color of the large-mouth morph was darker and had more uniform pigmentation and less mottling than the typical morph. This was especially marked along the ventral margin of the body (figure 2).

The large-mouth/broad-head morphology could have a developmental basis because the large-mouth morph becomes more distinctive with increasing body size. Study of small specimens will be necessary to determine whether allometric growth is involved in producing the large-mouth morph and at what stage of development this morphology becomes evident. Heterochronic processes involving changes in the timing of developmental events are suspected to have played a role in the divergence of some nototheniid species (Balushkin 1984), sometimes beginning in larval life (Klingenberg and Ekau 1996). Whether the morphological differences between the morphs of *T. newnesi* reflect underlying genetic differences is also unknown.

Proportional measurements and coloration suggest that the large-mouth morph leads a more benthic existence than the typical semipelagic morph. Having a blunt head and less streamlined body, the large-mouth morph possesses characteristics typically associated with
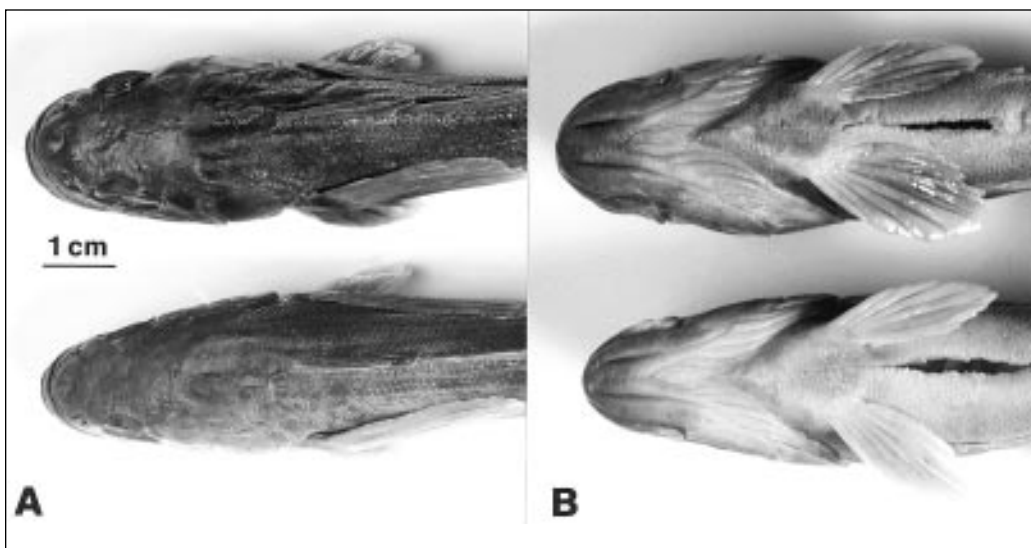

Figure 2. Dorsal (*A*) and ventral (*B*) views of same specimens shown in figure 1, with large-mouth morph on top. Broad U-shaped head of large-mouth morph is clearly shown. Head of typical morph is more tapered. Darker pigmentation pattern in large-mouth morph is also evident in ventral view. (× 1.1)

benthic morphs in other polymorphic fish (Skúlason and Smith 1995). In shallow areas free of anchor ice, the large-mouth morph of *T. newnesi* may live on the bottom as an ambush predator on both benthic and water column organisms. The large mouth and head allow the consumption of larger prey as well as a wider variety of prey. The typical morph of *T. newnesi* schools and feeds predominantly in the water column.

Distinct intraspecific morphs have appeared within a number of lineages of freshwater fishes, especially in low-diversity boreal lakes. At first glance, lakes appear to share few biological characteristics with the polar marine environment. Reduced competition associated with a low-diversity fish fauna, however, may be a common factor in the origin of intraspecific morphs in both lacustrine fishes and in *T. newnesi*. Trophic and morphological diversification predominates in species-poor fish communities, suggesting that the absence of competition allows niche expansion (Robinson and Wilson 1994).

Antarctic notothenioids are unusual among marine fishes in their ecological and morphological diversity and in their dominance of shelf and upper slope habitats within their range. The suborder Notothenioidei and the family Nototheniidae are adaptive radiations of about 120 and 50 species, respectively (Eastman 1993). The appearance of a large-mouth morph within *T. newnesi* is an additional small-scale radiation, and its discovery extends the bounds of the adaptive radiation of nototheniids to the intraspecific level. This finding suggests ecological and evolutionary parallels between the low-diversity ichthyofaunas of Arctic, boreal, and temperate lakes and of inshore waters of the high antarctic shelf. It implies that inshore antarctic waters are underused by fishes and a possible site of ongoing speciation.

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Temperature compensation of enzymatic activities in brain of antarctic fishes: Evidence for metabolic cold adaptation

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The extent to which metabolic rates of antarctic fishes are cold-adapted remains controversial (Holeton 1974; Clarke 1991). Several studies report that metabolic rates of antarctic fishes are higher than rates predicted for temperate or tropical fishes at the low-habitat temperatures of antarctic species (Wohlschlag 1960; Torres and Somero 1988a,b). At the level of whole-organism oxygen consumption, however, it is difficult to isolate effects of temperature adaptation from influences of other factors that affect metabolic rate, for example, locomotory habit, body size, and nutritional state. Certain of these factors may be more important than temperature in governing metabolic rate. Thus, as emphasized by Holeton (1974) and Clarke (1991), whole-organism oxygen consumption rates are unlikely to provide unambiguous conclusions about the extent of metabolic compensation to temperature.

To study temperature compensation in as unambiguous a manner as possible, we reasoned that measurement of the metabolic potential of an organ, brain, that performs the same functions in all fishes, regardless of adaptation temperature, locomotory habitat, phylogeny, or nutritional condition, could avoid the uncertainties inherent in studies of whole-organism respiration. Similarities in enzymatic activities in brains of temperate fishes with diverse locomotory habits (Somero and Childress 1980) suggested that, at a common adaptation temperature, only minimal variation in ATP-generating capacity of brain exists among fishes. In contrast to brain, and in support of the caveats raised by Holeton (1974) and Clarke (1991), activities of ATP-generating enzymes in white locomotory muscle vary by over a thousandfold among fish species with different capacities for locomotory activity (Somero 1992).

To examine metabolic potential of brain, we measured activities of enzymes indicative of aerobic and anaerobic pathways of ATP generation. Citrate synthase (CS) is an indicator of the level of mitochondrial citric acid cycle activity and, hence, of aerobic ATP-generating potential. Lactate dehydrogenase (LDH) is involved in anaerobic glycolysis and in conversion of lactate to pyruvate for subsequent oxidation in the citric acid cycle. Activities of CS and LDH, expressed as international units (IU) per gram wet weight of tissue, have been shown to correlate strongly with whole-organism oxygen consumption rates of fishes (Childress and Somero 1979; Torres and Somero 1988a,b). Thus, these enzymatic activities also should serve as excellent proxies for rates of tissue metabolism.

Comparison of CS and LDH activities in brains of antarctic nototheniid fishes collected in McMurdo Sound (water temperature near –1.86°C) and channichthyid fishes collected near Palmer Station (water temperature near –0.5 to 0°C) with activities in diverse temperate and tropical fishes revealed a high degree of metabolic compensation to temperature (figure). At a common temperature of measurement (10°C), CS (figure, block *A*) activities in brains of antarctic fishes were approximately 1.3–fold greater than activities in brains of temperate species (habitat temperatures of approximately 8–15°C) and 1.6–fold higher than in brains of tropical fishes (habitat temperatures of approximately 25–30°C). For LDH (figure, block *B*), differences in activity between antarctic fishes and the other two groups were even greater: activities in brains of antarctic fishes were 2.3–fold and 3.1–fold higher than those of temperate and tropical species, respectively.

In conjunction with the analyses of brain tissue, we measured CS and LDH activities in white and red locomotory muscles (data not shown). In agreement with other studies (Childress and Somero 1979; Somero 1992), enzymatic activities in red and white muscles varied widely among species, largely in reflection of interspecific differences in locomotory habit.

In summary, these comparisons of enzymatic activities speak directly to the central issues raised by Clarke (1991) and Holeton (1974) concerning the extent—indeed, even the reality—of metabolic compensation to temperature. The wide variation in enzymatic activity found in white locomotory muscle (Childress and Somero 1979; this study), the tissue constituting the largest percentage of a fish's mass, points out the difficulties inherent in distinguishing the effects of adaptation temperature on metabolic rate from effects due to locomotory activity and the costs of maintaining the skeletal muscle mass. When comparisons are made, however, using an organ such as brain in which metabolic functions are not apt to be linked to locomotory activity levels, distinguishing the extent of metabolic compensation to temperature can be done with minimum ambiguity.
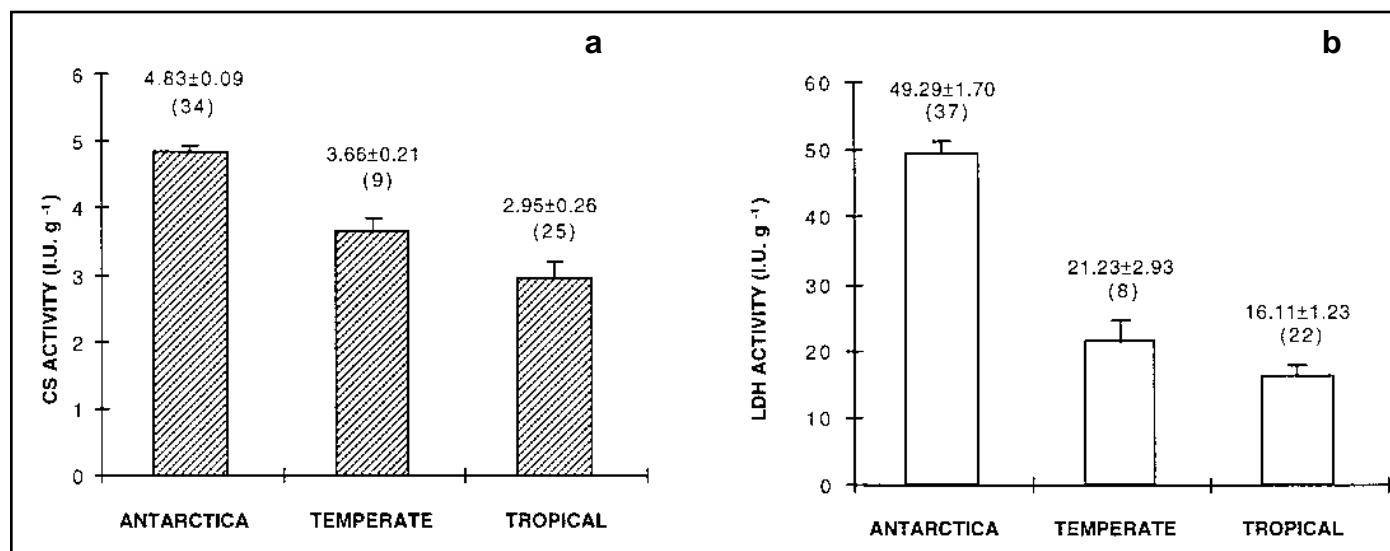
Activities of citrate synthase (CS) and lactate dehydrogenase (LDH) in brain tissue of antarctic, temperate, and tropical teleost fishes. Activities were measured at 10°C following protocols given in Torres and Somero (1988a), except that for the CS assays the concentration of dithionitrobenzoic acid was 1 millimolar (m*M*). Uncentrifuged homogenates were used. Activities are expressed as I.U. of activity (micromoles of substrate converted to product per minute) per gram wet mass of tissue. Duplicate measurements were made for each brain homogenate. The average values of CS and LDH activity for each brain were used to calculate the mean activities for each geographical group. The group means are given with the SEM and, in parentheses, the total number of brains analyzed for each geographical group. Variances were tested with Cochran's C test. In comparing mean CS activities (*A*), variances were unequal, so a Kruskal-Wallis analysis was applied. In *B*, one-way analysis of variance was applied for comparisons of means of LDH activities. In all comparisons of effect of habitat, significant differences ($p<0.05$) were observed for both enzymes. Antarctic species studied were *Pagothenia borchgrevinki, Trematomus bernacchii, Trematomus hansoni, Trematomus newnesi, Trematomus pennelli, Chaenocephalus aceratus, Champsocephalus gunnari,* and *Chianodraco rastrospinosus.* Temperate species studied were *Sebastes mystinus, Sebastes carnatus,* and *Leptocottus armatus.* Tropical species studied were *Halichoeres bivittatus, Halichoeres radiatus, Pomacentrus* sp., *Centropomus undecimalis,* and *Oreochromis* sp.

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Physiological energetics of "pelagic" and "demersal" development in the antarctic sea urchin *Sterechinus neumayeri*

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Thorson's rule has provided a focal point for numerous discussions of larval development in polar marine invertebrates (Thorson 1950). Initially, a developmental strategy of brooding was considered a requisite for larval survival in high latitudes, where low temperatures extend developmental times and patchy phytoplankton production restricts the availability of food for feeding larvae. It is now clear that brooded larvae are not a necessary life-history characteristic for species' persistence in polar environments (Pearse 1994, pp. 26–43) and that a wide range of developmental programs are exhibited by polar marine invertebrates (Bosch and Pearse 1990; Pearse, McClintock, and Bosch 1991).

For many antarctic echinoderms, it is unclear whether embryonic development is pelagic or demersal (Pearse and Bosch 1986). From our recent work with the antarctic sea urchin *Sterechinus neumayeri*, we have noted that spawned eggs are negatively buoyant and become entrained in a thick mucus layer when spawned. During the first 5 days of development (required to reach the hatching blastula stage at –1.5°C), embryos remain in this mucus aggregate if the cultures are not continuously mixed. In this study, we address whether any physiological or energetic processes are affected in *S. neumayeri* embryos if early development is "pelagic" (i.e., suspended in the water column) or "demersal" (here defined as undisturbed in an mucus aggregate).

Early development under pelagic and demersal conditions was assessed by culturing embryos to the hatching blastula stage using either

- stirring to keep them suspended in the water column ("Stir" denotes pelagic development) or

- no stirring to allow the embryos to remain aggregated in a mucus layer ("Still" denotes demersal development).

Both treatments used 500,000 fertilized eggs in 20-liter containers and 50 percent of the culture water was changed every 2 days.

Morphologically, hatching blastulae (HBs) of the demersal and pelagic culture treatments were significantly different. The demersal HBs had significantly thicker cell layers at the animal and vegetal poles (Mann-Whitney Rank Sum analysis of variance [ANOVA] $p<0.001$, $n=100$; figure 1*A*) with a corresponding significant reduction in blastocoel diameter (figure 1*A*). Despite the increase in cell thickness, however, the overall size of the HBs did not differ between culture treatments (figure 1*A*).

The differences in thickness of the embryonic layers between treatments were paralleled by significant changes in organic mass. The demersal cultured HBs had a significantly higher ash-free, dry, organic mass (AFDM) than the pelagic cultured HBs (ANOVA $p<0.01$, Tukey's test $p<0.05$, $n=10$; figure 1*B*). The magnitude of this mass increase was dramatic, representing a 19 percent increase over the pelagic HBs and a total increase of 24 percent over the initial AFDM of the eggs. Thus, if the developing embryos of *S. neumayeri* are left undisturbed, they have a strong potential to obtain exogenous organic material from their immediate environment, presumably via transport of substrates from the jelly-coat or other secondary mucus materials released with the eggs at spawning.

To characterize the differences in HBs further, we measured the total uptake of sulfur-35 (^{35}S) methionine from filtered sea water (0.2-micrometer, FSW) and the subsequent incorporation of radioactive label into protein. The amount of

^{35}S-methionine in protein was measured as the radioactive counts that were precipitable by trichloroacetic acid (5 percent). Half the HBs were sampled after a 12-hour exposure to the isotope (50,000 HBs and 500 microcuries in 50 milliliters FSW); the remaining HBs were transferred to FSW without any isotope for an additional 36 hours before sampling again (total time, 48 hours). The demersal cultured HBs had accumulated a significantly larger amount of methionine from FSW after 12 hours (ANOVA, $p<0.003$; Tukey's test, $p<0.05$; $n=3$; figure 2A). At the 48-hour time point, total amounts of ^{35}S-methionine remained significantly higher in the demersal HBs; no significant decrease occurred between 12 and 48 hours (analysis as above; figure 2A). A corresponding significantly higher incorporation of ^{35}S-methionine into protein in the demersal HBs was noted at 12 hours, and this higher level serves as an indicator of an increased protein synthesis rate. At 48 hours, this significant increase in protein synthesis was still evident (analysis as above; figure 2B).

Thus, early development in *S. neumayeri* is significantly affected by whether or not embryos are stirred in a culture container or allowed to settle to the bottom in a mucus aggregate. Although these experimental treatments are only approximations of the conditions embryos might experience if they were advected into the water column (pelagic development) or entrained on the bottom (demersal development), it is evident that the immediate availability of mucus or jelly-coat materials does have a significant effect on the morphology and physiology of embryogenesis.
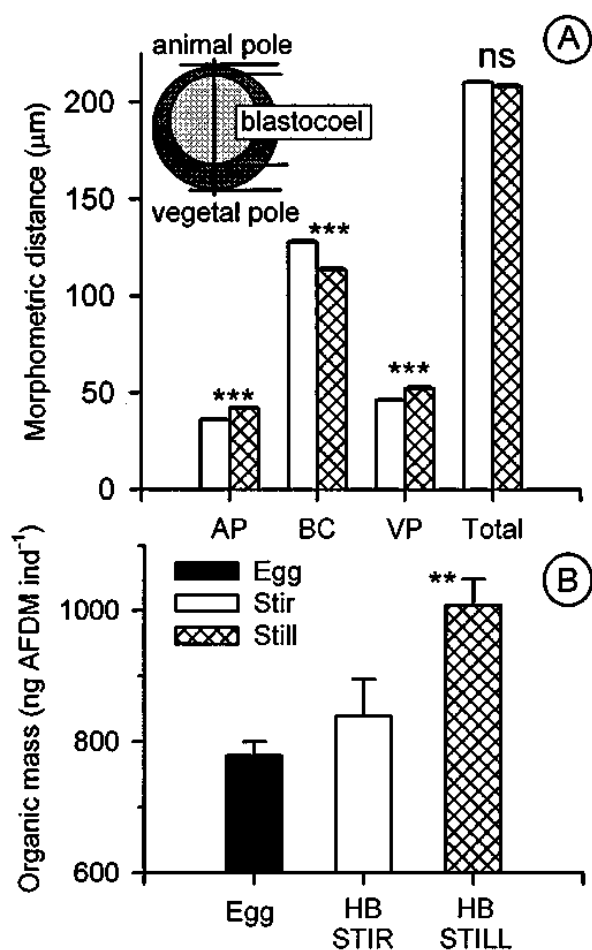
Figure 1. *Sterechinus neumayeri* size and mass differences for hatching blastulae cultured with either stirring (open histogram denotes "Stir") or no stirring (cross-hatch histogram denotes "Still"). *A*. Morphometry of hatching blastulae dimensions (mean ±1 standard error of the mean, $n=100$). (AP = animal pole thickness; BC = blastocoel diameter; VP = vegetal pole thickness; *** = significant differences between Still and Stir treatments [$p<0.001$]; ns = not significant.) *B*. Organic mass of eggs (solid histogram) and hatching blastulae (mean ±1 standard error of the mean, $n=10$). (** = significant differences between Still and Stir treatments [$p<0.01$]; ng = nanograms; ind^{-1} = per individual).
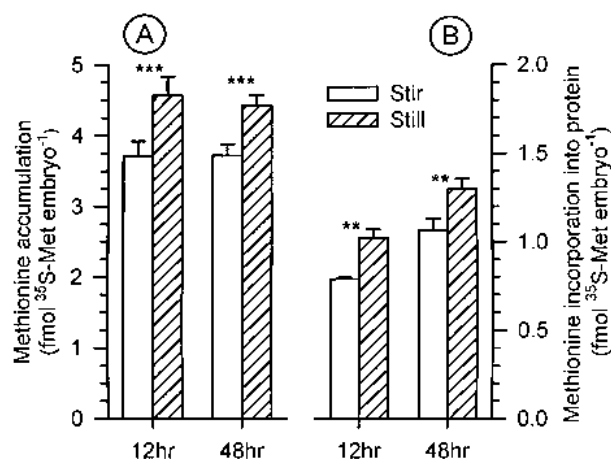

Figure 2. *Sterechinus neumayeri* accumulation of ^{35}S-methionine and its subsequent incorporation into proteins in hatching blastulae cultured with either stirring (open histograms denote "Stir") or no stirring (cross-hatch histograms denote "Still"). Each histogram represents a mean ±1 standard error of the mean; $n=3$. *A*. Total accumulation of ^{35}S-methionine after a 12-hour exposure to the isotope in filtered sea water (FSW) and later after a transfer to FSW without isotope (48 hour). (*** = significant differences between Still and Stir treatments [$p<0.001$]). *B*. Amount of ^{35}S-methionine incorporated into newly synthesized proteins during the same time course as above. (** = significant differences between Still and Stir treatments [$p<0.003$]; fmole = femtomole; embryo^{-1} = per embryo.)

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The kinetics of the cortical reaction and respiratory burst following fertilization of *Sterechinus neumayeri* eggs

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The fertilization events in eggs of temperate sea urchins have been well studied and the early phases, such as those associated with the cortical reaction, are initiated between 30 and 60 seconds after fertilization and lead to the elevation and hardening of the fertilization envelope (Paul and Epel 1971; Heinecke and Shapiro 1992). The earlier period between sperm–egg binding and the cortical reaction is referred to as the latent period, and presumably, changes evoked by the sperm during this time interval lead to a calcium rise and initiation of the cortical reaction (*see* Whitaker, Swann, and Crossley 1987, pp. 157–172).

This article describes two fertilization-associated events in the antarctic urchin, *Sterechinus neumayeri* specifically

- the timing of the onset of the cortical reaction as a function of temperature and
- the respiratory burst associated with the production of hydrogen peroxide used in the hardening of this fertilization envelope (*see* Heinecke and Shapiro 1992).

Adult *S. neumayeri* were collected from the waters of McMurdo Sound and maintained in running sea water at approximately –1°C. Spawning was induced by intracoelomic injection of 0.5-molar potassium chloride, viable eggs were pooled, and the resulting embryos and larvae were cultured following the techniques of Shilling and Manahan (1994).

The onset of the cortical reaction was determined by videotaping a suspension of eggs on a microscope slide at varying temperatures maintained (between –2°C and 12°C) with a piezoelectric stage cooler. Fifty microliters of an egg suspension was placed on the slide for 5 minutes to equilibrate, and then 5 microliters of sperm suspension were added with mixing. The images were videotaped through a Leitz microscope at 160 × magnification such that one egg was viewed and recorded for 5–10 minutes at the indicated temperatures. The videotape replayed, allowing determination of initial sperm attachment and start of the cortical reaction. The timing of the initiation phase was assessed, only using eggs where the envelope elevation could be seen as a blister arising from the equator of the egg and then progressing around the egg surface. As seen in figure 1, the elevation of the fertilization envelope (i.e., cortical reaction) began at 60 seconds at 12°C, at 135 seconds at 4°C, and at 177 seconds at –1.8°C.

To measure respiration during fertilization, between 400 and 700 eggs were placed in chilled (–1°C) microrespiration chambers (300 microliters total volume) and allowed to equilibrate for 1 hour. A dilute sperm mixture (1:50,000 concentration) was then carefully injected into the chamber with a Hamilton syringe. To ensure synchronous fertilization after sperm injection and even mixing during respiration measurements, a tiny magnetic stir bar was added to the chamber and periodically rotated by hand using a large external magnet. Fertilization varied between 45 and 95 percent, with different batches of eggs. Oxygen concentration within the chamber was measured using a polarographic microelectrode and oxygen meter (*see* Jaeckle and Manahan 1992 and Podolsky et al. 1994).

As previously shown in temperate species (Ohnishi and Sugiyama 1963; Epel 1964; Yasumasu et al. 1988), a distinct increase in oxygen consumption occurs after fertilization. This "respiratory burst" began about 3 minutes postfertilization (figure 2), was maximum for the next 2–3 minutes, and then gradually decreased to a stable, postfertilization level about two times greater than in unfertilized eggs. The relative rates of respiration during the burst and 15 minutes after fertilization were 7.2±3.1 standard deviation (n=8) and 2.1±0.56 standard deviation (n=8) times greater than that of the unfertilized eggs, respectively. The respiratory rates of the early embryos were similar to those reported for this antarctic species (Podolsky et al. 1994, data not shown) and other echinoids (i.e., Ohnishi and Sugiyama 1963).

The timing of these early fertilization events in *S. neumayeri* at ambient antarctic temperature (–1°C) was significantly slower than that seen in temperate sea urchins. In *Strongylocentrotus purpuratus* at 16°C the cortical reaction is first apparent 30 seconds after insemination and the respiratory burst follows about 15–30 seconds later (Epel 1964; Paul and Epel 1971). This near coincidence of the beginning of the respiratory burst and the cortical reaction is also seen in *S.*
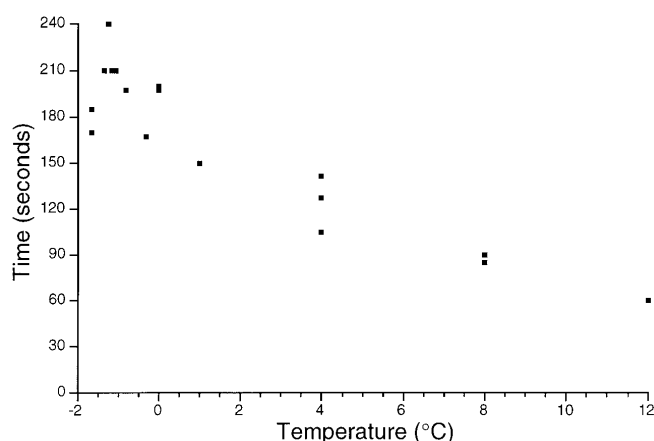

Figure 1. Time until the initiation of fertilization membrane elevation after the introduction of sperm at different temperatures in *S. neumayeri* eggs.

neumayeri, but the timing of the change is much later (about 3 minutes at –1°C).

Although these results indicate that the fertilization processes are similar in temperate and antarctic forms, an important difference is the retardation of the events at these low temperatures. Sperm bind quickly to the eggs, but the latent period (*see* Whitaker et al. 1989, pp. 157–172) is significantly delayed.

The latent period of the fertilization process is poorly understood, and the protracted nature of this phase in the antarctic urchins could provide new insights into this important stage that eventually leads to the postfertilization calcium rise and egg activation. Further studies on fertilization in these polar forms are also needed to determine whether the block to polyspermy (see Jaffe and Gould 1985, pp. 223–251) is similarly retarded or whether this occurs early and the other sequelae are the only ones with this significant lag period.
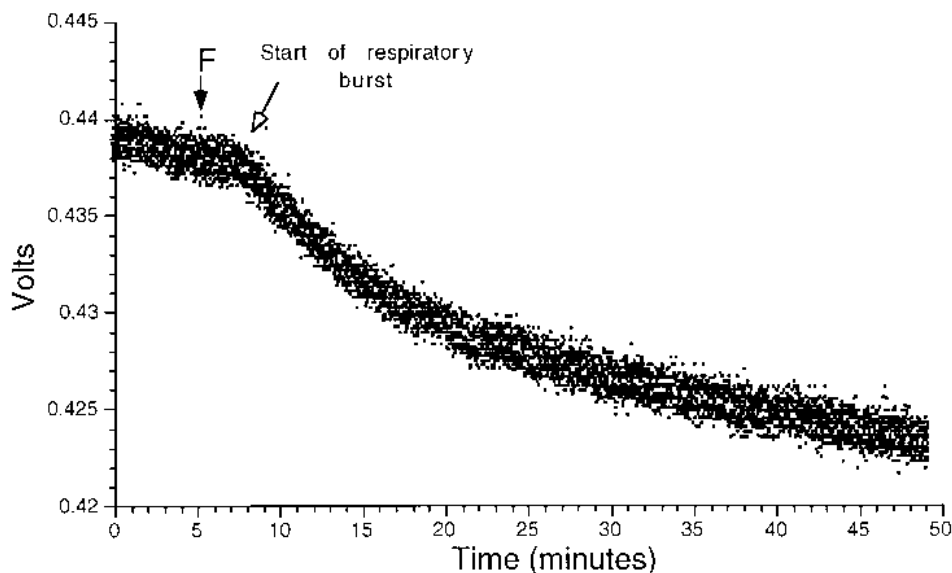

Figure 2. Sample trace of polarographic oxygen electrode voltage before and after fertilization of *S. neumayeri* eggs. The change in electrode voltage is proportional to oxygen consumption within the microrespirometry chamber. All tests were done at –1°C. The "F" indicates the time when sperm was injected into the chamber and the open arrow indicates the approximate initiation of the respiratory burst.

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Changes during development in activities of the sodium/potassium-ATPase enzyme in the antarctic sea urchin *Sterechinus neumayeri*

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The sodium/potassium (Na^+,K^+)-ATPase enzyme is a transmembrane protein that maintains ion gradients in animal cells. The energy required to operate this "ion pump" is high, accounting for over 20 percent of the metabolic rate of animal tissues (Milligan and McBride 1985). Because of its central metabolic role, the regulation of this enzyme is important in adaptational strategies such as physiological responses to low oxygen and low temperature (Hochachka 1986, 1988).

As part of a study to understand energy metabolism during development of the antarctic sea urchin *Sterechinus neumayeri*, we measured the change in activity of Na^+,K^+-ATPase during development of embryos to the feeding larval stage (pluteus). Fertilized eggs were reared through embryogenesis in 20-liter vessels containing ambient McMurdo Sound sea water that had been passed through a 0.2-micrometer pore-size filter. The cultures were maintained in the aquarium at McMurdo Station at –1°C and the culture water was changed every 4 to 5 days. Embryos and larvae were harvested from these cultures for determination of Na^+,K^+-ATPase activities over the first 22 days of development, until the first feeding larval stage was reached.

The physiological activity of Na^+,K^+-ATPase was determined in living embryos and larvae (herein, referred to as "*in vivo*" measurements) by measuring their transport rates at –1°C of rubidium-86 ($^{86}Rb^+$), an analog commonly used as a tracer of K^+ flux (Hilden and Hokin 1975). The transport rate of K^+ in developing sea urchins that was attributable to Na^+,K^+-ATPase only (*cf.* general K^+ flux) was measured as the difference in K^+ transport rates in the presence and absence of ouabain, a specific inhibitor for Na^+,K^+-ATPase. The total amount of enzyme activity (herein, referred to as "*in vitro*" measurements) was also measured in tissue homogenates of the same stages of development that were studied for the *in vivo* measurements. Total *in vitro* Na^+,K^+-ATPase activity was measured according to Esmann (1988) with the following modifications

- the Na^+,K^+-ATPase of *S. neumayeri* was not very sensitive to ouabain and a concentration of 14–16 millimolar (m*M*) (figure 1*A*) was required to inhibit all the activity;
- the optimal pH for the enzyme assay was found to be from pH 8 to 8.3.

All *in vitro* assays were conducted at 15°C, the temperature found in preliminary experiments to give a measurable signal above background in the enzyme assay. The activity of the enzyme observed at this higher temperature was converted to activity-equivalents at the animals' ambient temperature (–1°C) for comparisons of total (*in vitro*) and physiological active (*in vivo*) enzyme levels. In our preliminary experiments, the effect of temperature on Na^+,K^+-ATPase activity was described by a Q_{10} value of 2.3. This value was used for the above conversion of enzyme activity at 15°C to a rate at –1°C.

Figure 1*B* shows that the activity of total *in vitro* Na^+,K^+-ATPase remained constant from soon after fertilization (day 0) to the gastrula stage at day 9 (by analysis of variance, the variance ratio [VR] = 0.08^{ns}, $F_{0.05[1,8]}$=5.32; where ns = not significant). Later stages of development had higher activity levels of
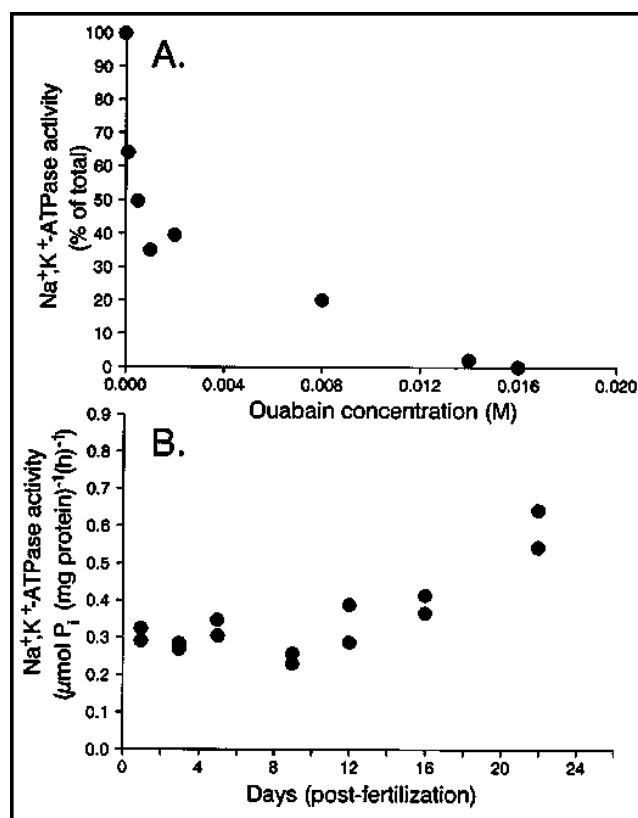

Figure 1. *A.* Ouabain inhibition of Na^+,K^+-ATPase activity in *Sterechinus neumayeri*. Concentrations of ouabain tested ranged from 10^{-9} *M* to 16×10^{-3} *M*. Complete inhibition of ouabain-sensitive activity of Na^+,K^+-ATPase occurred at around 14 m*M* ouabain. Each data point represents the mean of two or three replicates measured at 15°C on homogenates of the early pluteus larval stage. *B.* Change in the total activity of Na^+,K^+-ATPase during embryological development to the larval stage. The seven developmental stages at which total Na^+,K^+-ATPase activity was measured were the fertilized egg (day 1), the early morula (day 3), the hatching blastula (day 5), the early gastrula (day 9), the late gastrula (day 12), the prism larva (day 16), and the early pluteus larva (day 22). Each data point represents the mean of five or six replicates (at 15°C). [μmol denotes micromole; (mg protein)$^{-1}$ denotes milligram per protein; (h)$^{-1}$ denotes per hour.]

the enzyme. The increase during later development of total protein-specific Na$^+$,K$^+$-ATPase activity was not an artifact of a developmental change in protein content because no significant change in the amount of protein per individual was noted (VR=0.08^{ns}, $F_{0.05[1,40]}$=4.08). Figure 2 gives a comparison of the total and the *in vivo* Na$^+$,K$^+$-ATPase activities. A range of 27 to 86 percent of the total enzyme activities was physiologically active, depending on the stage of development.

The total protein-specific Na$^+$,K$^+$-ATPase activities reported here for developing stages of the antarctic sea urchin (*S. neumayeri*) are approximately half of those we have measured (Leong and Manahan, unpublished data) for similar stages of development in two temperate species of sea urchin (from California, *Strongylocentrotus purpuratus* and *Lytechinus pictus*). Considering the metabolic "cost" of high Na$^+$,K$^+$-ATPase activities in animals, the lower specific activities we report here for *S. neumayeri* suggest that these antarctic embryos have a lower energy requirement for ion regulation.

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Figure 2. Comparison of total (solid bars) and *in vivo* (open bars) Na$^+$,K$^+$-ATPase activities through early development of *Sterechinus neumayeri*. Each solid bar is the mean of two assays; error bars are one standard error of the mean. Each open bar is the mean with error for two experiments measuring the *in vivo* rate of K$^+$ transport. The K$^+$ transport rate for each of the two experiments was calculated from time-course experiments of ^{86}Rb$^+$ flux, each of which had 8 to 9 individual data points that were used to calculate a rate. The number above each pair of bars for a given stage of development represents the percent of the total Na$^+$,K$^+$-ATPase activity that was active *in vivo* at the corresponding developmental stage (days postfertilization). [μmol denotes micromole; (mg protein)$^{-1}$ denotes milligram per protein; (h)$^{-1}$ denotes per hour.]

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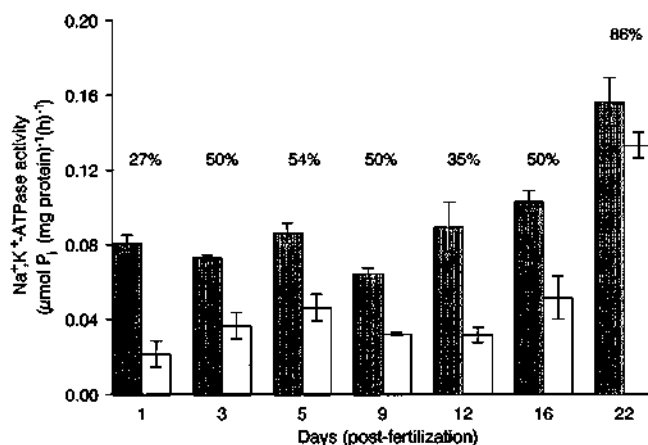
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Occurrence of *Gromia oviformis* in McMurdo Sound

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A species of large, testate sarcodine is a conspicuous component of the shallow benthos in McMurdo Sound and elsewhere in the Antarctic. On the basis of its general appearance, this protist was previously identified as *Gromia cf. oviformis* (Filosea:Protista) (Heron-Allen and Earland 1922; DeLaca 1986; Kiest 1993; Gooday, Bowser, and Bernhard in press). As noted by Jepps (1926) and Arnold (1982), however, gross morphological characters to differentiate potential congeners within this obscure group are rare; detailed autecologic information is also lacking.

To confirm the identity of this protist, we examined test (shell) ultrastructure in specimens collected from Explorers Cove, western McMurdo Sound, and off McMurdo Station on the eastern side of the Sound. Specimens were chemically fixed and embedded for transmission electron microscopy using our standard methods (*see*, for example, Bowser et al. 1995). The embedded material was cut into serial 250-nanometer (nm) thick sections, stained with uranyl acetate and lead citrate, and viewed with an AEI EM7 high-voltage electron microscope (HVEM) operated at 1.0 megaelectron-

volts (MeV), or a JEOL JEM-4000FX intermediate-voltage electron microscope (IVEM) operated at 0.4 MeV. Survey micrographs of the proteinaceous test revealed laminations similar to those observed in *Gromia oviformis* collected in temperate coastal waters (Hedley and Wakefield 1969; Arnold 1982). Plan views of the shell material show the "honeycombed membrane" as described for *G. oviformis* by Hedley and Wakefield (1969). This unusual structure comprises a hexagonal array of cylinders with an 18-nm center-to-center spacing, interconnected by electron-opaque septae (figure 1). To the best of our knowledge, these ultrastructural features are unique to *G. oviformis* Dujardin, thus confirming its occurrence in McMurdo Sound. Furthermore, our thick-section approach has revealed new features of the honeycombed membrane, e.g., the density in the cylinder lumen (arrows in figure 1). This feature persists after lattice filtration of the images, as seen here in the high-pass filtered image (figure 1B). The superimposition of lightly stained repeating details in thick sections (Rieder 1979; Bowser et al. 1995) revealed this feature in our study. The crystalline structure of the honeycombed membrane may prove to be a useful test object for magnification calibration and distortion assessment in microscopy. In addition, the material properties of the honeycomb membrane afforded by its uniform hexagonal architecture are amenable to biomechanical analyses using finite-element methods; such studies may yield clues to the ecophysiological significance of this structure and may also find applications in nanoengineering.

As noted in previous studies, *Gromia oviformis* is an abundant and conspicuous epifaunal component of the benthos in McMurdo Sound (figure 2; see also Oliver and Slattery 1985). For example, in December 1990, their densities ranged from 500 to 89,000 per square meter ($\bar{x}$=35,000;

n=46; sd=20,000) on rocky substrate at a water depth of 15 meters just south of McMurdo Station. In Explorers Cove, located on the relatively oligotrophic western side of McMurdo Sound, we consistently observed lower densities (e.g., 1,357 per square meter in November 1993; table 9 in Gooday et al. 1996). In addition, specimens in the cove appear much smaller and distinctly ovoid compared with the spherical specimens off McMurdo Station (personal observations). Certain shallow areas in the cove, however—for example, adjacent to underwater ice cliffs—had high densities of relatively large, spherical *G. oviformis*. Such densities are likely due to higher concentrations of organic matter in these areas. We speculate that the density and morphology of *G. oviformis* reflect local productivity. Because this protist is easy to quantify and collect in the field and manipulate in the laboratory, it might prove to be a useful model system in antarctic ecosystem studies, for example, effects of eutrophication on benthic meiofauna.
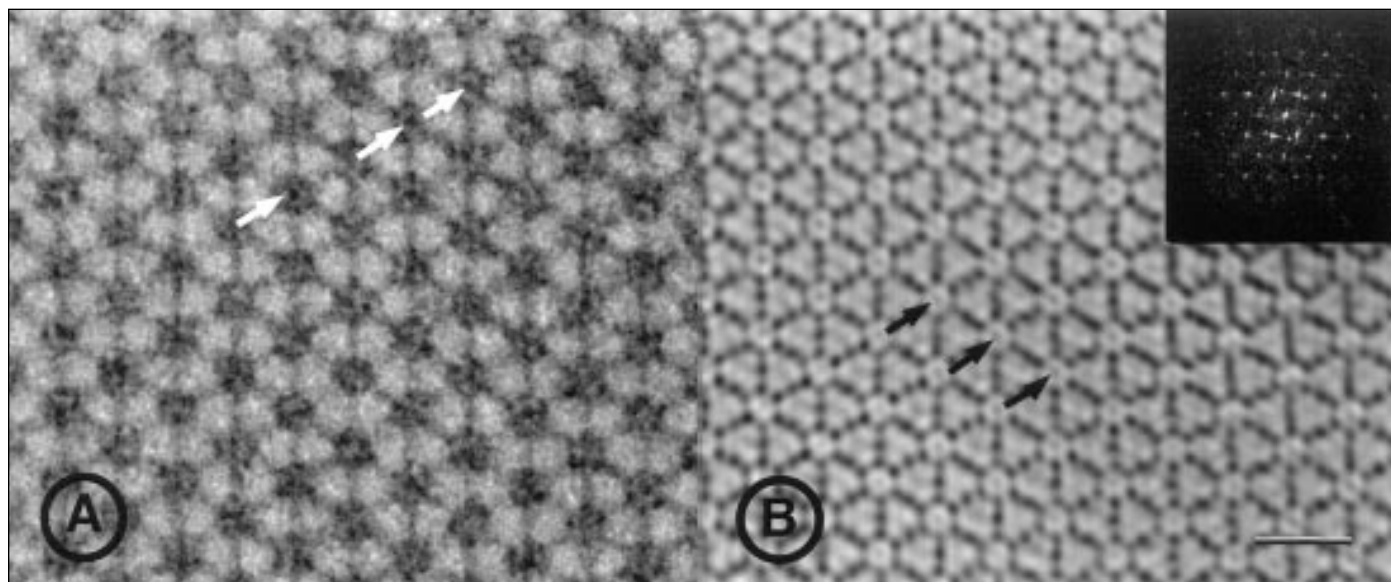
Figure 1. Intermediate-voltage electron micrographs of a 250-nm-thick tangential section through *Gromia oviformis*, showing the "honeycombed membrane" layer of the test. Unprocessed (*A*) and digitally filtered (*B*) images reveal new features, such as the density at the center of the hexagonal repeating unit (arrows). Inset: Corresponding diffraction pattern used to make the spatial mask. Bar = 25 nm.
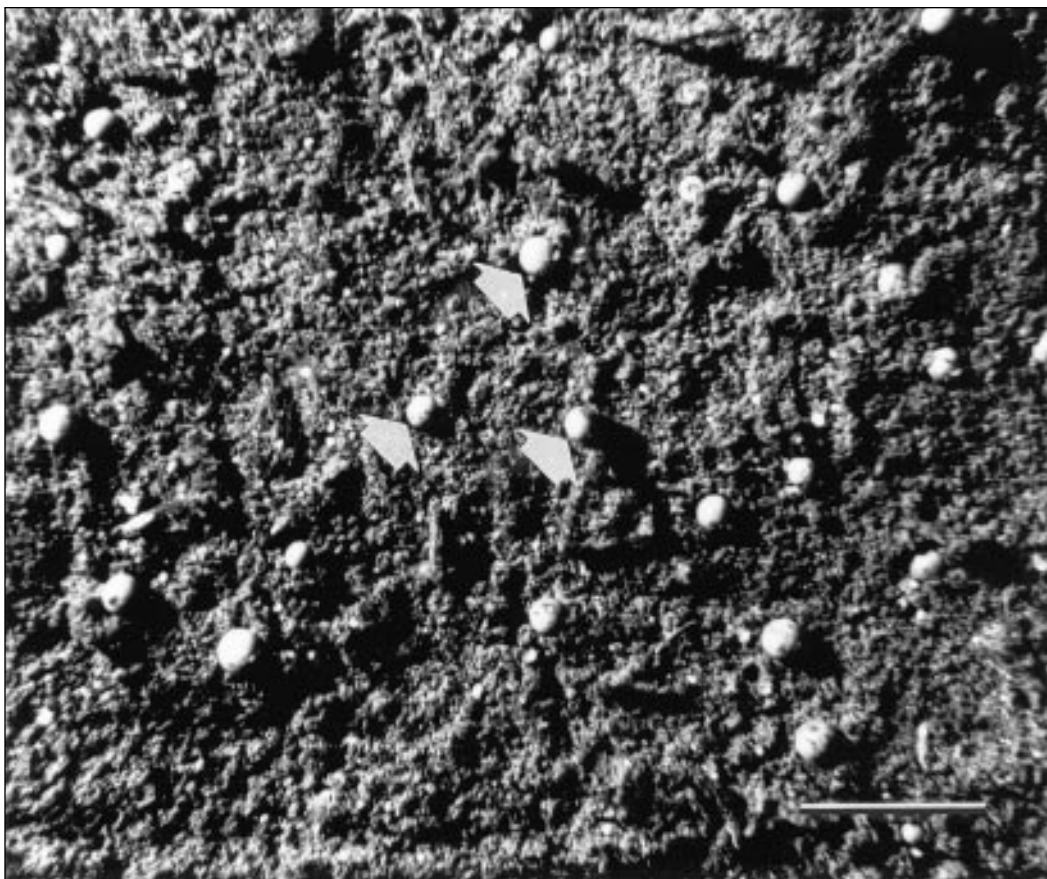
Figure 2. Macro photograph of the McMurdo collection site, showing the typical abundance of *Gromia oviformis* (e.g., arrows) on shallow, rocky substrates. Bar = 1 cm.

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